Electronic Supplementary Information (ESI)

Micro- and nano-tubules built from loosely- and tightly-rolled up thin sheets

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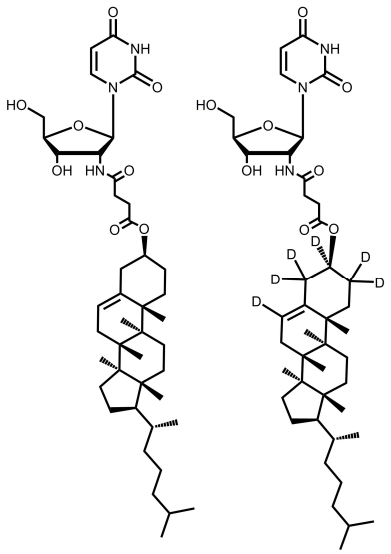
Synthesis of *2'-N-(2-(Cholester-1,2,3,4,4,6-D6-yl)-succinyl)-2'-deoxy-2'-aminouridine.* Partially deuterated CholAU was synthesized in analogy to the non-deuterated compound by reaction of 2-dexoxy-2-amino-5′-trityluridine with the partly deuterated cholesteryl succinate. The latter was obtained by following the chemical procedure described in the literature (Y.-L. Yang, G.-L. Chan, X.-J. Ma, K. Deng, Y.-T. Shen, X.-Z, Feng, C. Wang *Angew. Chem. Int. ed*. **2006**, 45, 6889) starting from a partially deuterated cholesterol precursor (2,2,3,4,4,6-*d*6, 97-98%, Eurisotop, Saarbrücken, Germany). After purification by column chromatography 78% yield was obtained and used for 2′-amide formation. 2-Desoxy-2-amino-5′-trityluridine (0.213 g, 0.00044 mol), the partially deuterated cholesteryl succinate (0.198 g, 0.0004 mol), EDC (0.08 g, 0.00052 mol), HOBt (0.081 g, 0.0006 mol) and DIPEA (0.056 g, 0.00044 mol) were dissolved in 20 ml dry DCM under argon atmosphere, and the reaction mixture was stirred at room temperature for 26 h. The crude reaction mixture was purified by column chromatography using DCM:MeOH = 10:1 as eluent, giving 0.30 g of the 5′-trityl-protected product as a yellow-white solid with 78 % yield. HRMS (ESI): m/z [M+1]- for $C_{59}H_{69}D_6N_3O_8$, found 982.5824 [M+Na^+].

5′-Deprotection. The trityl protected uracil-cholesterol derivative (0.20 g) was deprotected with trifluoroacetic acid (catalytic amount) in dichloromethane (3 mL). The reaction mixture was stirred at room temperature for 4 h. Then the solvent was immediately removed under vacuum and the remainder was purified by column chromatography with DCM:MeOH = 10:1 as eluent to give 0.085 g of a yellow-white solid (58% yield). HRMS (ESI): m/z [M+1]- for $C_{40}H_{55}D_6N_3O_8$, found 740.4716 [M+Na^+].



Scheme S1. Chemical structures of 2'-*N*-(2-(cholesteryl)-succinyl)-2'-desoxy-2'-aminouridine (CholAU) and the deuterated analogue 2'-*N*-(2-(Cholester-1,2,3,4,4,6-D6-yl)-succinyl)-2'-deoxy-2'-aminouridine (CholAU-d_6).

Pure CholAU precipitates in water

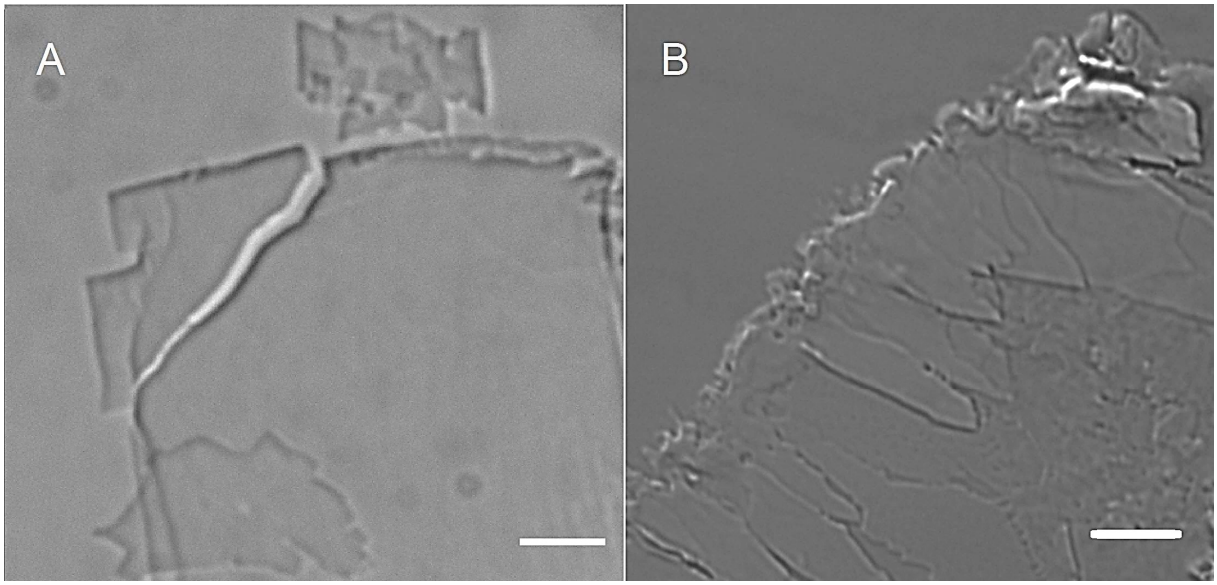
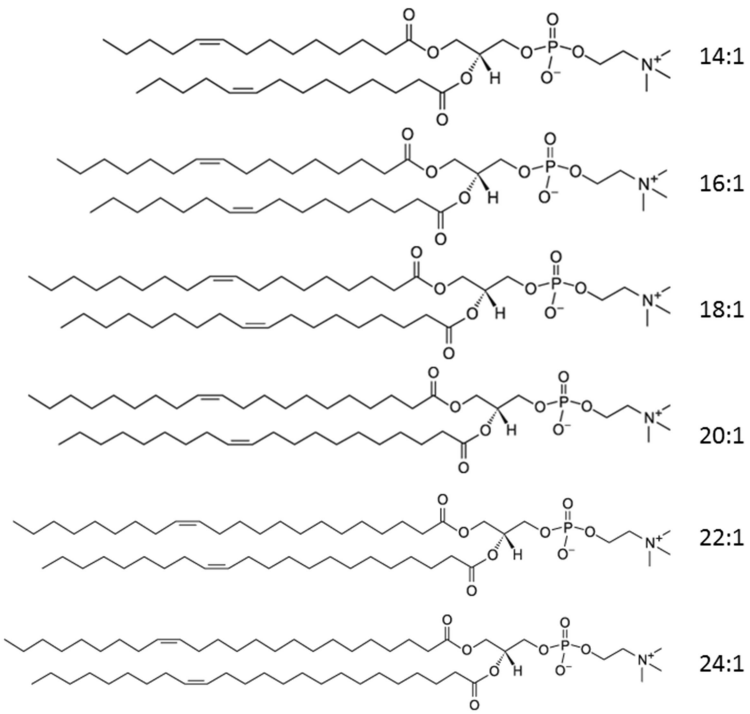


Figure S1. (A) CholAU solution in methanol (5 mg/ml) was injected into 70°C water. After cooling the suspension big crystals of CholAU were observed. (B) Cholesterol solution in methanol was injected into 70°C water. After cooling the suspension big crystals of cholesterol were observed. Differential interference contrast images. Scale bars correspond to 5 μm.

Dependence of the tubular assembly on the length of unsaturated PC chain



Scheme S2. Chemical structures of the phosphatidylcholines used (Avanti Polar Lipids (Alabaster, AL)).

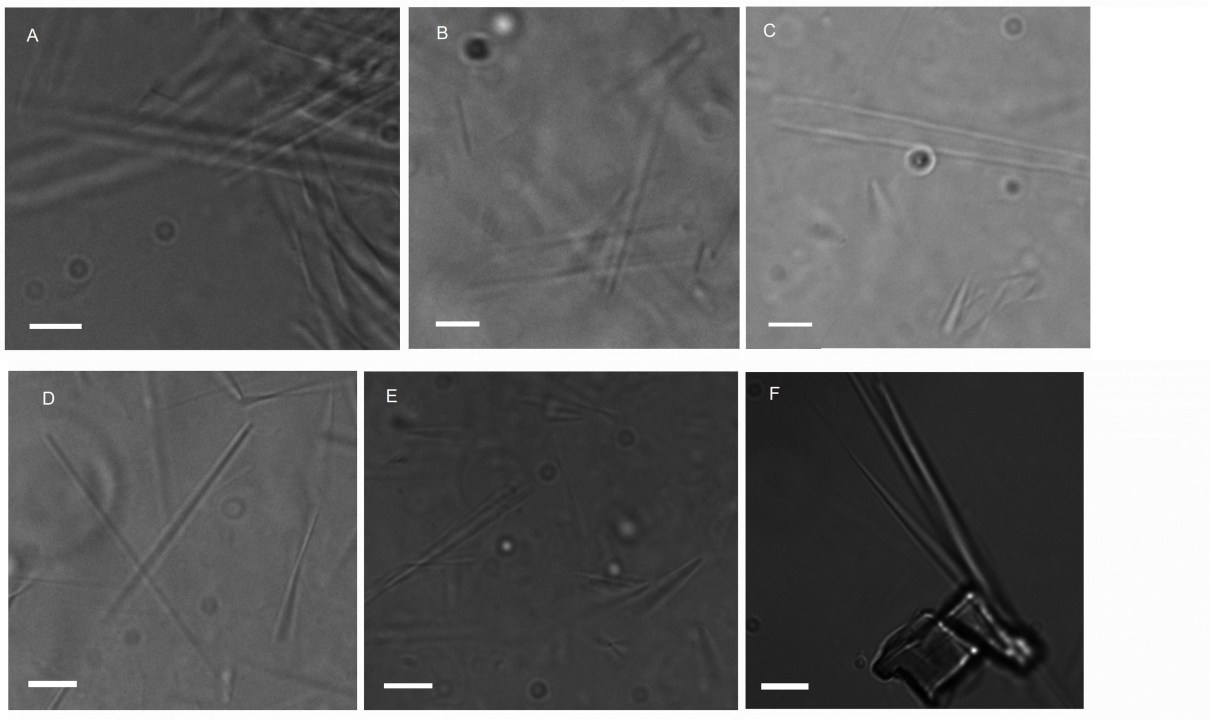


Figure S2. Dependence of the tubular self-assembly on the chain length of the unsaturated phosphatidylcholine. Differential interference contrast images for binary mixtures of CholAU and (A) 14:1, (B) 16:1, (C) 18:1 PCs revealing micro- and nano-tubules. Differential interference contrast images for binary mixtures of CholAU and (D) 20:1, (E) 22:1, (F) 24:1 revealing only nano-tubules and, additionally, crystals in F. Scale bars correspond to 5 μm.

Enlarged Figure 1A

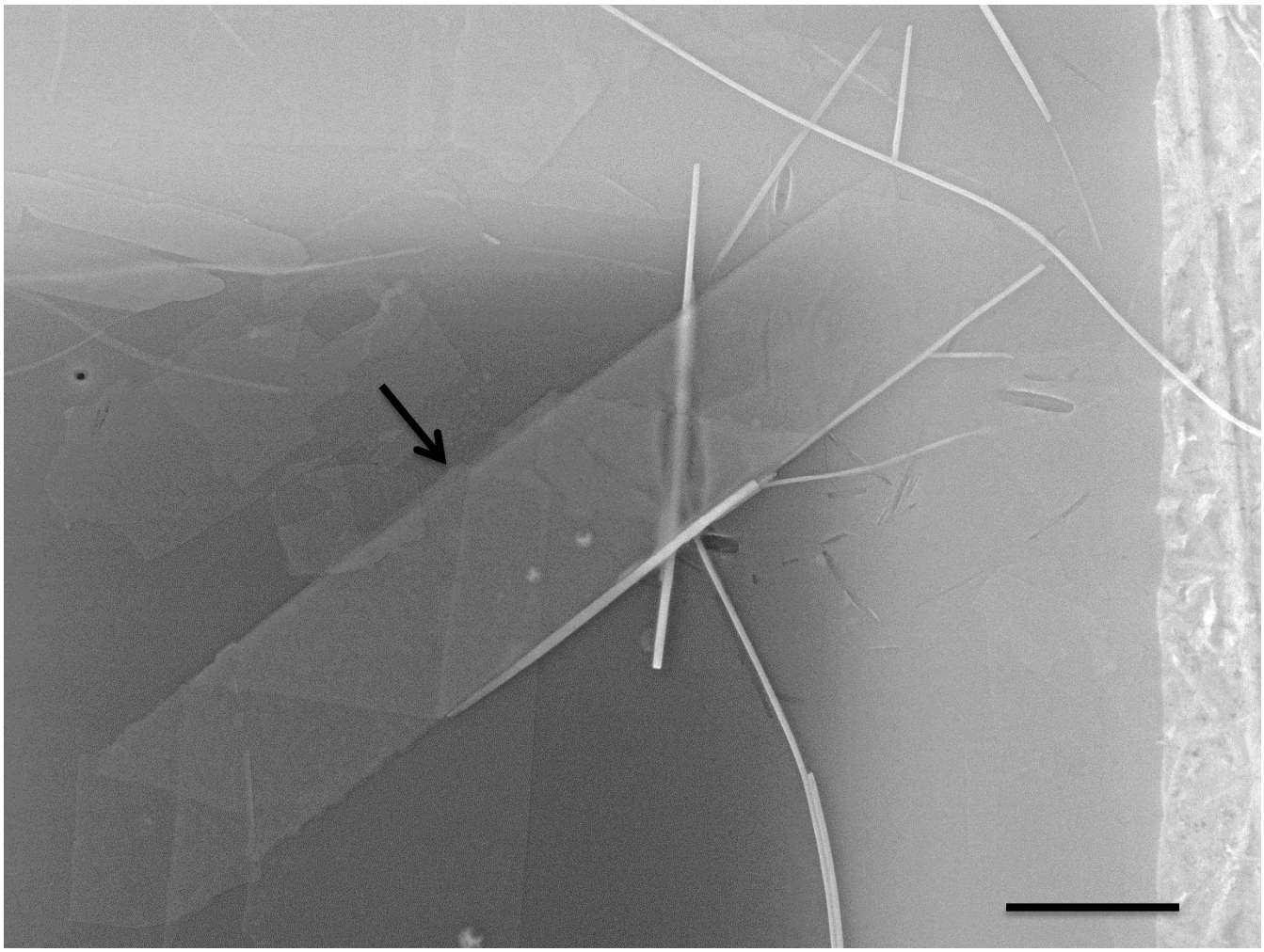


Figure 1A (enlarged). Typical self-assembled structures, e.g. nano-tubules and flat sheets, in a CholAU/DMoPC sample imaged by scanning electron microscopy (SEM). The flat structure resembles a folded sheet of paper with helical markings indicated with an arrow. The scale bar represents 5 µm.

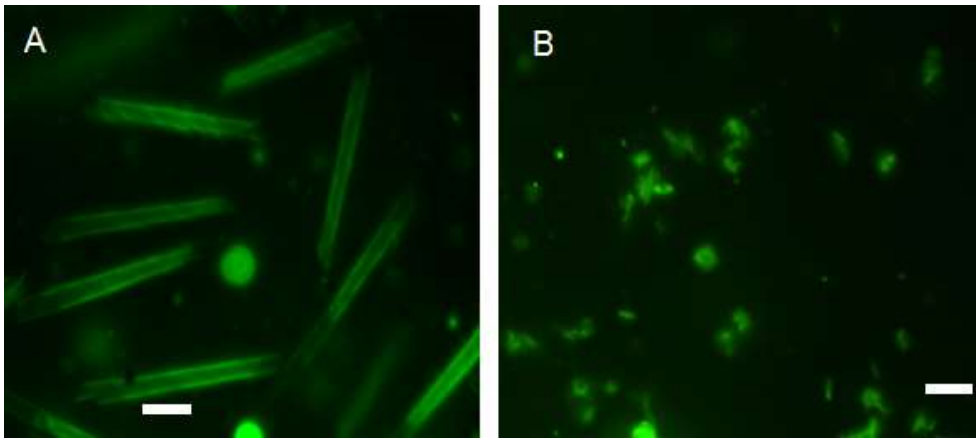


Figure S3. (A) CholAU/DMoPC micro-tubules formed upon the cooling of the 40:59.5:0.5 CholAU/DMoPC/NBD-DMPE aqueos suspension from 70 °C to RT. (B) Addition of 5 mM mβCD led to a gradual dissociation of the micro-tubules, 2 h after addition. Scale bars correspond to 5 µm.

Transmission electron microscopy of CholAU/DMoPC and CholAU/DOPC nano-tubules

A) B)

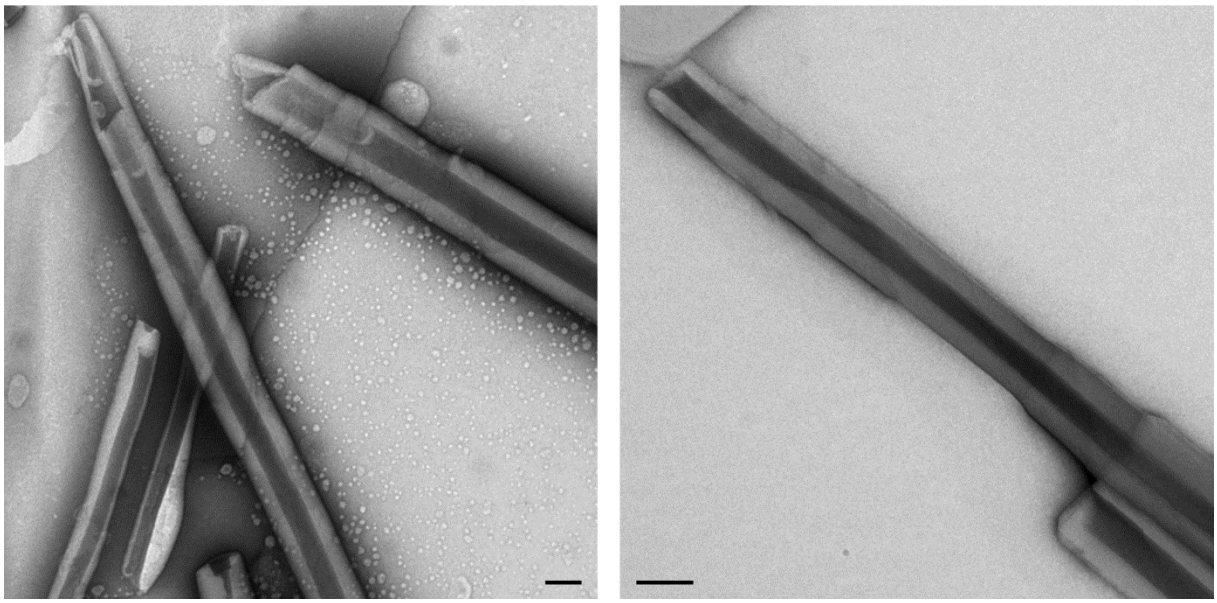


Figure S4. Negative staining TEM images of nano-tubules formed from 59.5:40:0.5 DMoPC:CholAU:NBD-DMPE mol% (A) and 69.5:30:0.5 DOPC:CholAU:NBD-DPPE mol% (B). Scale bars correspond to 100 nm.

Cryo scanning electron microscopy

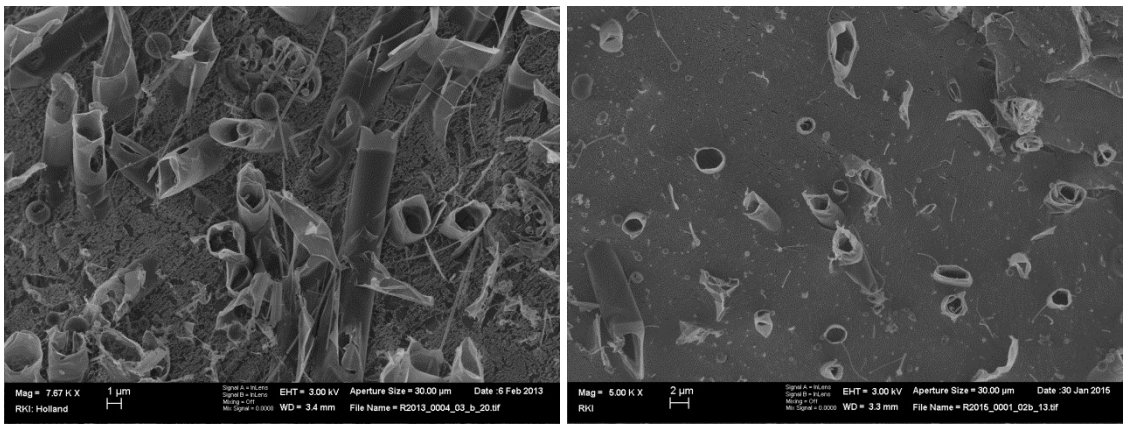


Figure S5. Cryo-SEM overview images of CholAU/DMoPC (left) and CholAU/DOPC (right) samples. Scale bars are 1 µm and 2 µm, respectively.

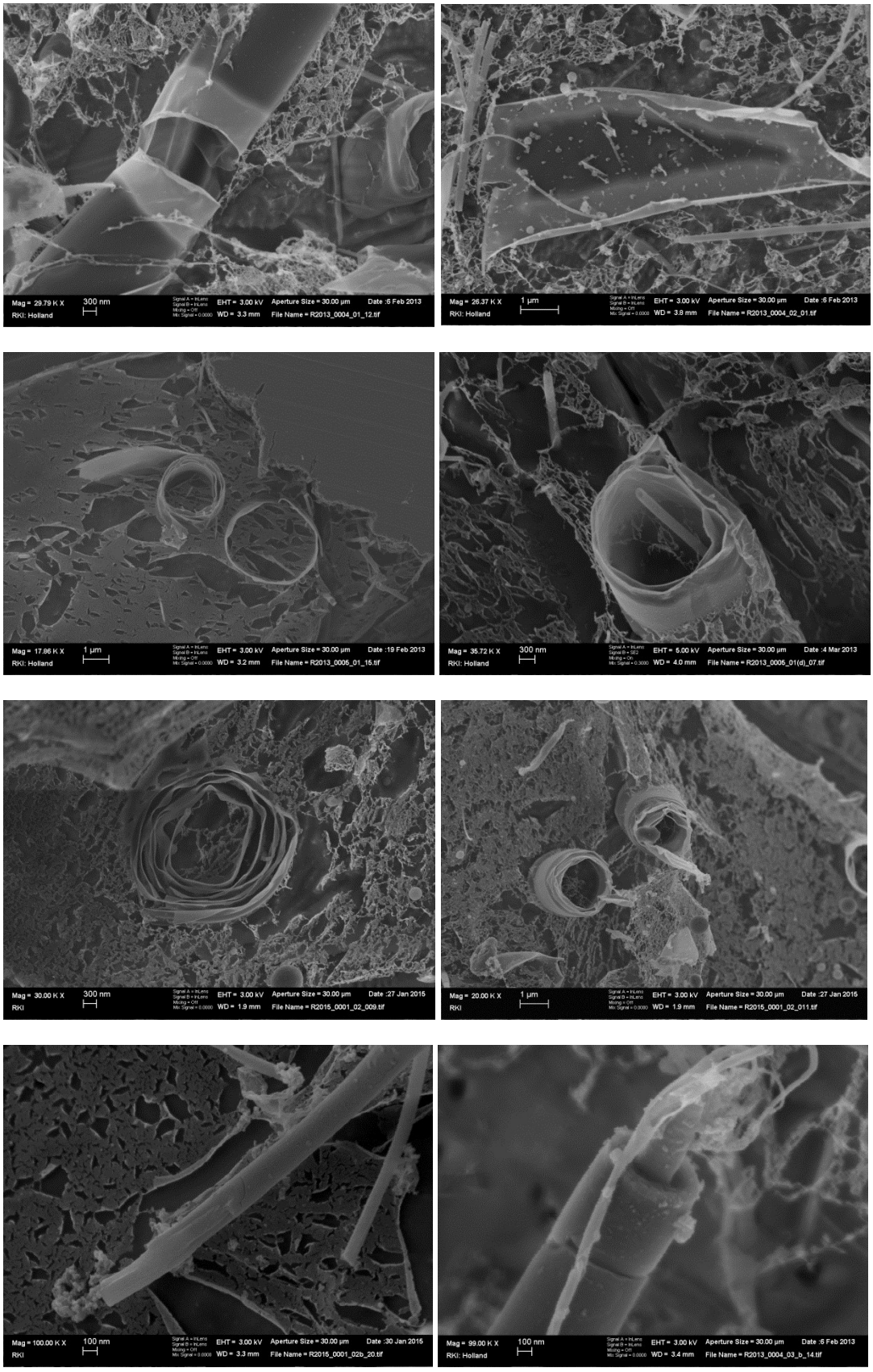


Figure S6. Cryo-SEM images of CholAU/DMoPC (left) and CholAU/DOPC (right) samples revealing micro-, nano-tubules, and sheets.

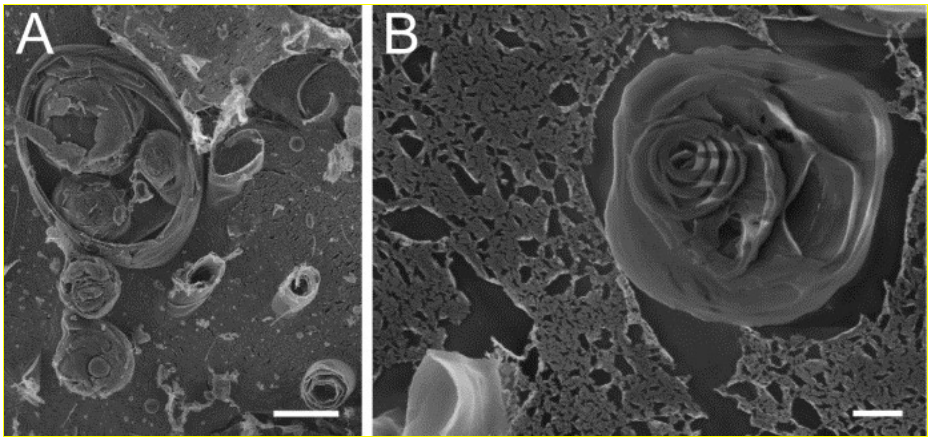


Figure S7. Cryo-SEM images revealed also phospholipid-characteristic vesicles in CholAU/DOPC (A) and CholAU/DMoPC (B) samples. Scale bars correspond to 5µm and 500 nm for (A) and (B), respectively.

AFM topology images

59.5:40:0.5 DMoPC:CholAU:NBD-DMPE or 59:40:0.5:0.5 DMoPC:CholAU:NBD-DMPE:DHE mol% mixtures were used to prepare the samples for AFM.

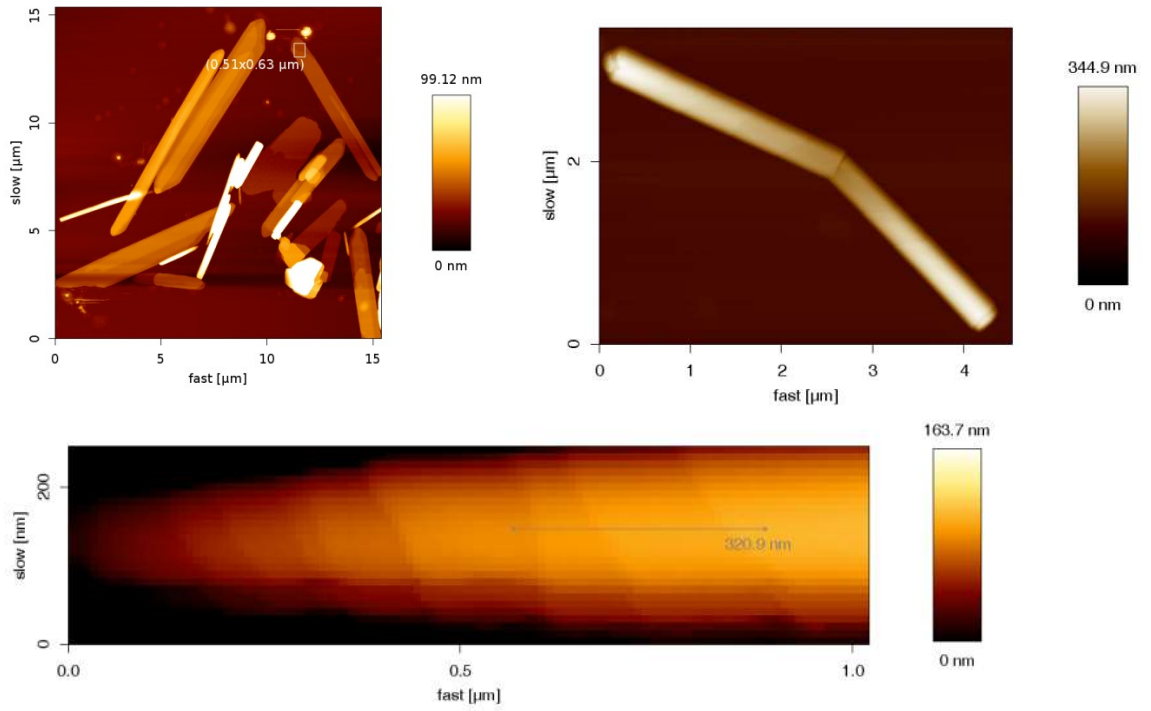


Figure S8. AFM topology images of flat and tubular structures in air-dried CholAU/DMoPC samples.

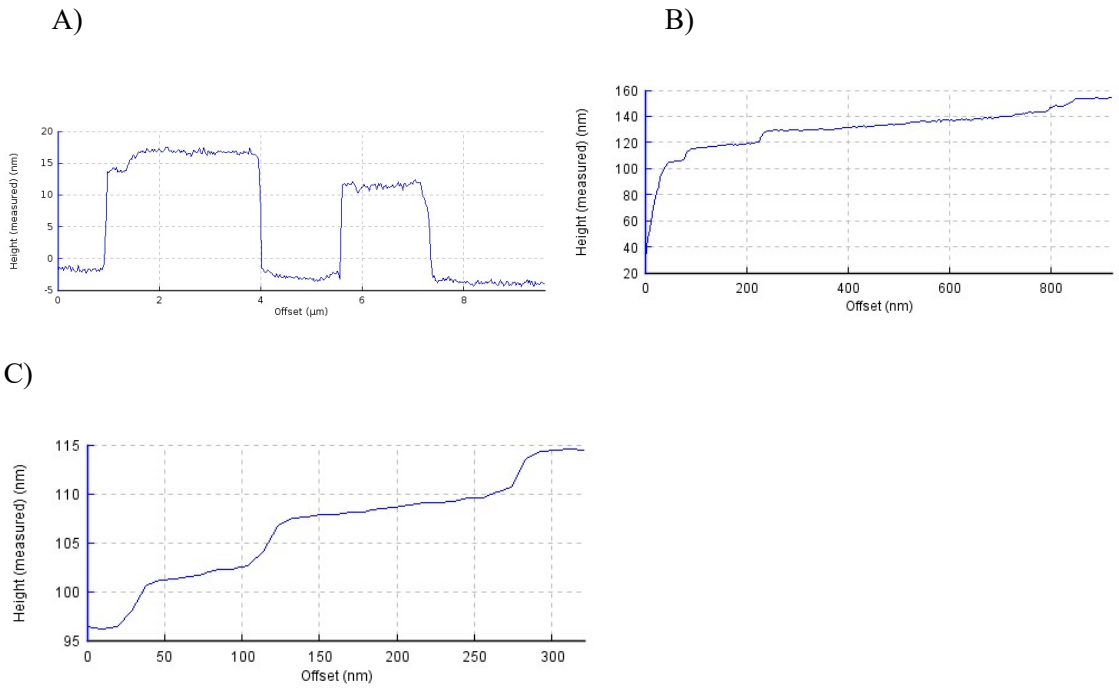


Figure S9. Examples of height cross sections for the structures in air-dried CholAU/DMoPC samples of A) flat structures, B) right end of the tubule shown in Fig. 4B. C) The cross section corresponds to the line shown in bottom image of Fig. S6. Note the complete topography image of this tubule is shown in Fig. 4C

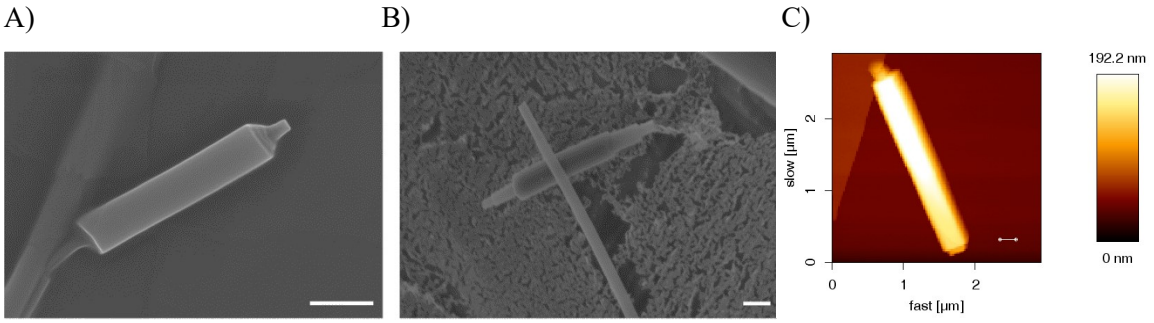


Figure S10. CholAU/DMoPC nano-tubules; RT-SEM (A), cryo-SEM (B), and AFM (C) images revealing very similar nano-tubular structures. Scale bars correspond to 500 nm.

AFM topography image of cholesterol crystals on mica

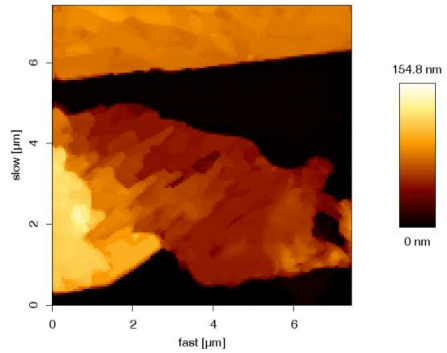


Figure S11. AFM topography map of pure cholesterol crystals on mica.

Nano-tubules scrolled from lamellar sheets

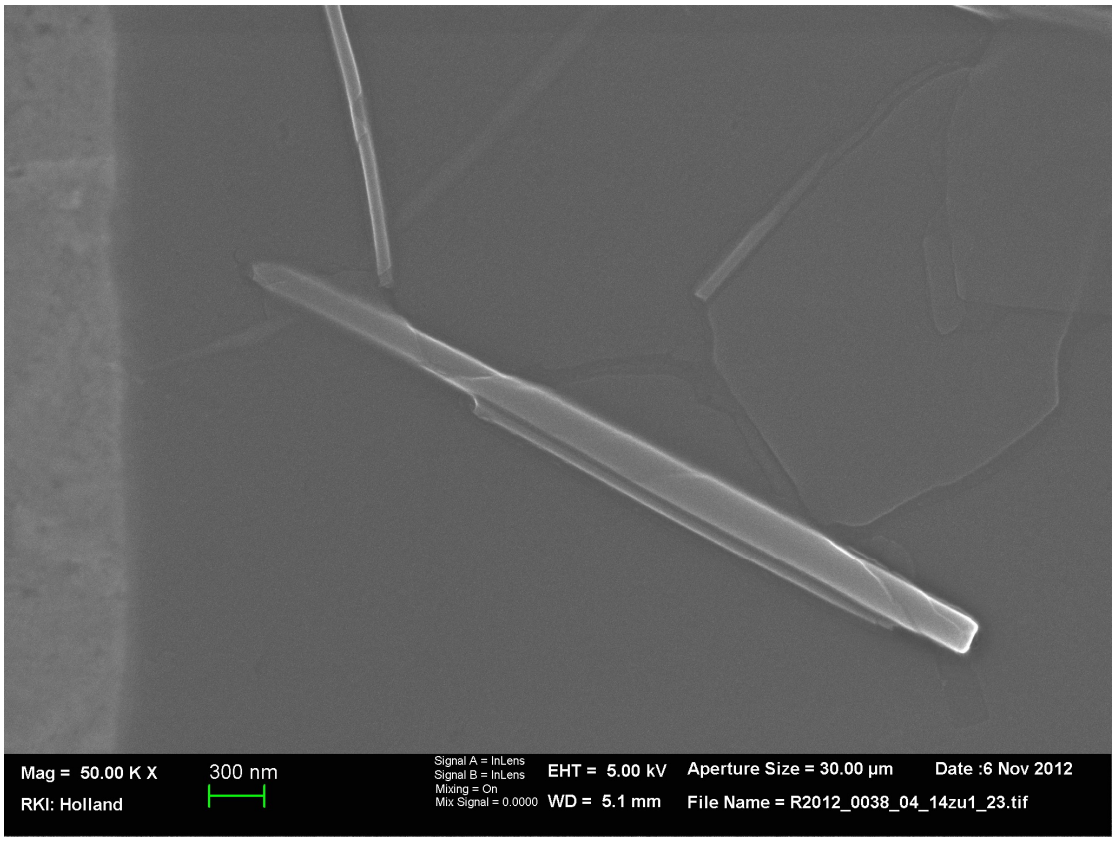
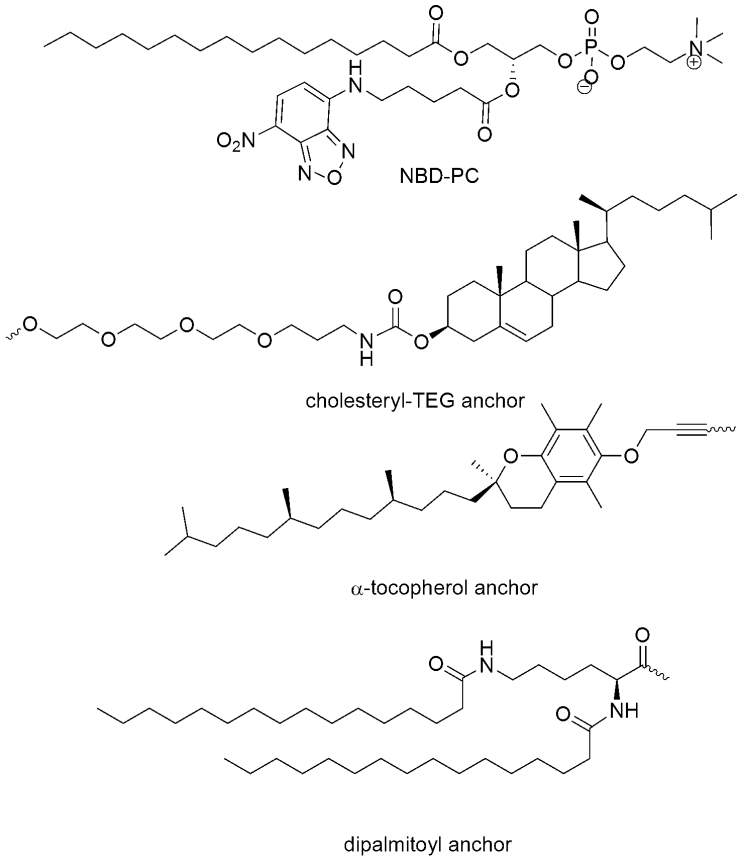


Figure S12. Room temperature SEM image of nano-tubules and a flat sheet assembled in a CholAU/DMoPC sample. Scale bar corresponds to 300 nm. Note the outer sheet of the nano-tubule in the middle was partially unwrapped.

Functionalization of tubules with lipophilic conjugates



Scheme S3. Chemical structure of C6-NBD-PC and the lipophilic anchors of the used lipophilic nucleic acids.

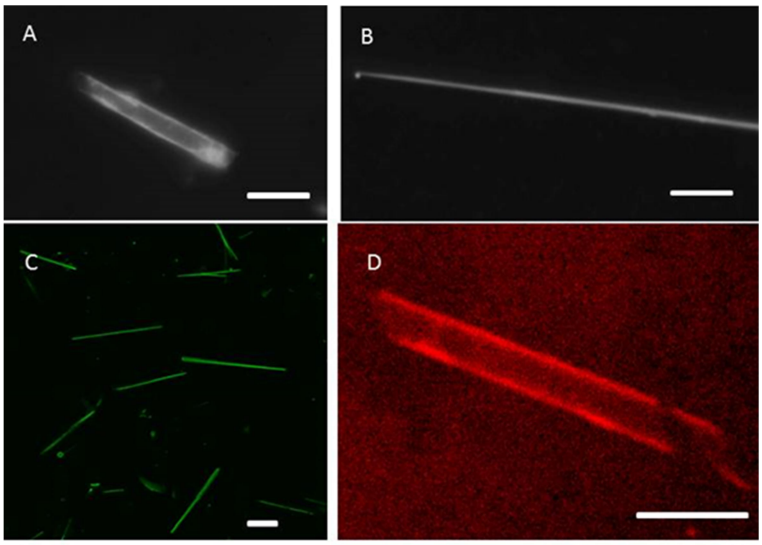
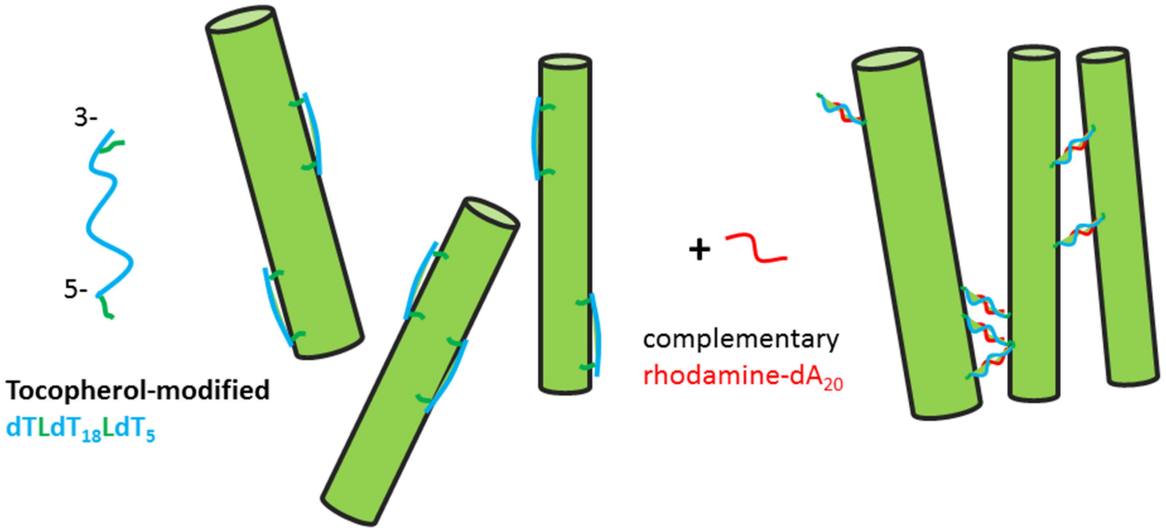


Figure S13. A, B) Fluorescence images of C6-NBD-PC labeled CholAU/DOPC micro- and nano-tubules. C) Fluorescence image of CholAU/DOPC nano-tubules functionalized with cholesteryl-TEG modified oligonucleotides and FAM-labeled complementary strand with following sequences: cholesteryl-TEG-5'-TCC GTC GTG CCT TAT TTC TGA TGT CCA-3', 5'-AGG CAC GAC GGA-3'-cholesteryl-TEG, 5'-GGT ACA ACT AAG ATA-3'-FAM. FAM fluorescence is shown in green. D) Confocal image of a micro-tubule functionalized with 200 nM double palmitoylated PNA (Pal-Lys(Pal)-Gly-Glu-Glu-Gly - ttc ttc tcc tt – Glu-Glu-Gly-CONH2) for 30 min, then 200 nM rhodamine-

labeled complement (AAG GAG AAG AAT – rhodamine , red) was added. Scale bars correspond to 5 µm. Confocal microscopy images (Figures S13 C, D and S15, right) were acquired using an inverted confocal laser scanning microscope FluoView 1000 (Olympus, Hamburg, Germany) with a 60x (NA 1.2) water-immersion objective. NBD (FAM), and rhodamine were excited with a 488 nm Argon laser and a 559 nm He-Ne laser, and detected sequentially at 500 nm – 545 nm and 570 nm – 630 nm, respectively.

Cross-linking of micro-tubules upon hybridization of lipophilic DNA with tocopherol anchors close to the ends with complementary strand.



Scheme S4 illustrates cross-linking of micro-tubules induced by lipophilic DNA (blue) modified with two anchors (green) upon hybridization with complementary strand (red). The scheme is not to scale. If only lipophilic DNA is present, both anchors incorporate into one tubule. It is assumed that addition of a complementary strand, 3´Rh$(dA)_{20}$, led to the formation of a rigid DNA helix between the anchors; as a consequence one of the α-tocopherol anchors was pulled out of the tubule, where the anchor was incorporated before, and could insert into the outer leaflet of another tubule. Similar cross-linking of lipid vesicles was described before (U. Jakobsen, A. C. Simonsen, and S. Vogel, J. Am. Chem. Soc., 2008, 130, 10462–10463; G. Zhang, F. Farooqui, O. Kinstler, and R. L. Letsinger, Tetrahedron Lett., 1996, 37, 6243–6246; D. Serien, C. Grimm, J. Liebscher, A. Herrmann and A. Arbuzova, *New J. Chem.*, 2014, **38**, 5181–5185).

NBD fluorescence **rhodamine fluorescence**

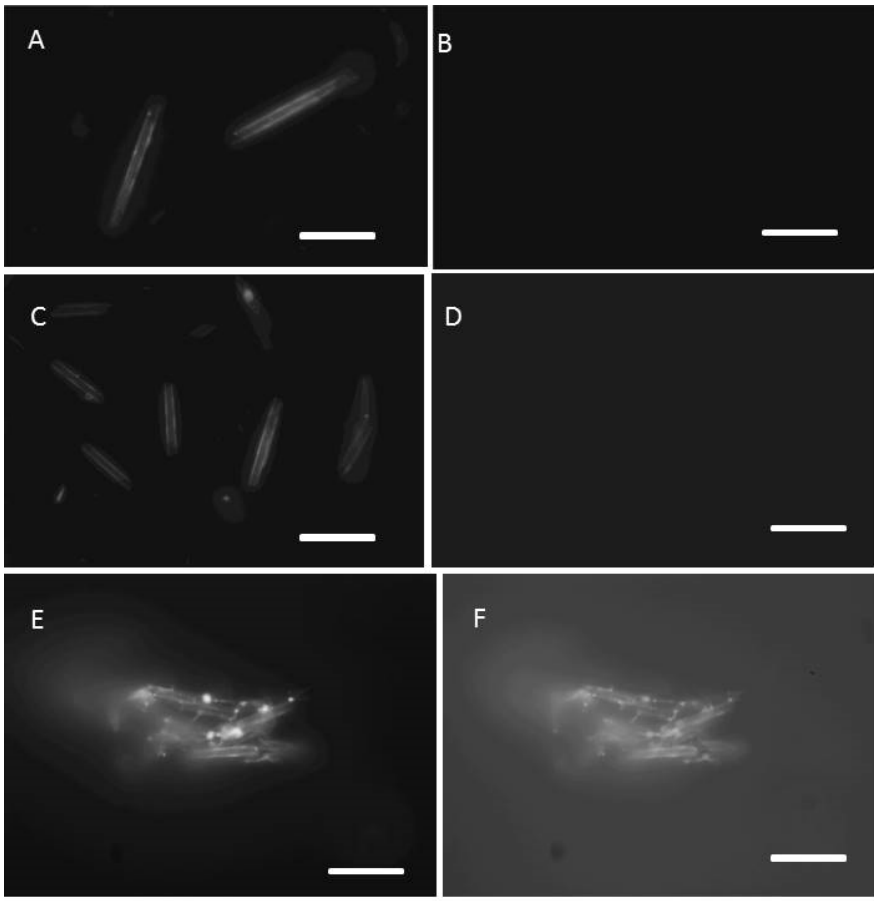


Figure S14. A, B) CholAU/DOPC/NBD-PE micro-tubules incubated with 200 nM tocopherol-modified DNA oligonucleotide $dTLdT_{18}LdT_5$, where L is tocopherol anchor shown in Scheme S1 in 150 mM NaCl, 10 mM HEPES, 1 mM EDTA, pH 7.4 buffer at room temperature for 5 min. Then 200 nM complementary rhodamine-labeled dA_{20} oligonucleotides were added. Non-specific binding of rhodamine-labeled dA_{20} was not detected (C, D). After (E, E) initiating the cross-linking of the tubules functionalized with the hybrid of the lipophilic DNA with dA_{20} by centrifugation in a swing rotor for 15 min at 1000 g clusters of tubules were observed. (A, C, E) NBD fluorescence; (B, D, F) rhodamine fluorescence. Scale bars correspond to 10 μm. All images were taken with an inverted IX-81 fluorescence microscope (Olympus, Tokyo, Japan) and a 60× (N.A. 1.35) oil-immersion objective using U-MWNiba (BP470-495, BA520IF, DM510-550) and U-MNG2 (BP572-612, DM 562) filter sets for green and red fluorophores, respectively, and Andor Clara interline CCD camera (Andor Technology Ltd., UK) at 25°C. Note, upon incubation at 37°C for one hour allowing dehybridization and rearrangement and cooling to room temperature, even bigger clusters of the tubules were observed. Note, when tubules were incubated with either of the oligonucleotides only isolated tubules were observed (A, C). Addition of a non-complementary strand neither induced any aggregation of the tubules. The rhodamine-labeled non-complementary strands, dT_{20}, were also distributed homogeneously in the presence and in the absence of the tocopherol-modified oligonucleotide and tubules. For more technical details on the tocopherol-modified DNA and cross-linking see D. Serien, C. Grimm, J. Liebscher, A. Herrmann and A. Arbuzova, *New J. Chem.*, 2014, **38**, 5181–5185; Kurz, A.; Bunge, A.; Windeck, A. K.; Rost, M.; Flasche, W.; Arbuzova, A.; Strohbach, D.; Müller, S.; Liebscher, J.; Huster, D.; and Herrmann, A. *Angew. Chem., Int. Ed.* **2006**, *45*, 4440–4444; Loew, M.; Kang, J.; Dähne, L.; Hendus-Altenburger, R.; Kaczmarek, O.; Liebscher, J.; Huster, D.; Ludwig, K.; Böttcher, C.; Herrmann, and A.; Arbuzova, A. Small 2009, 5, 320–323.

Loading of the tubules

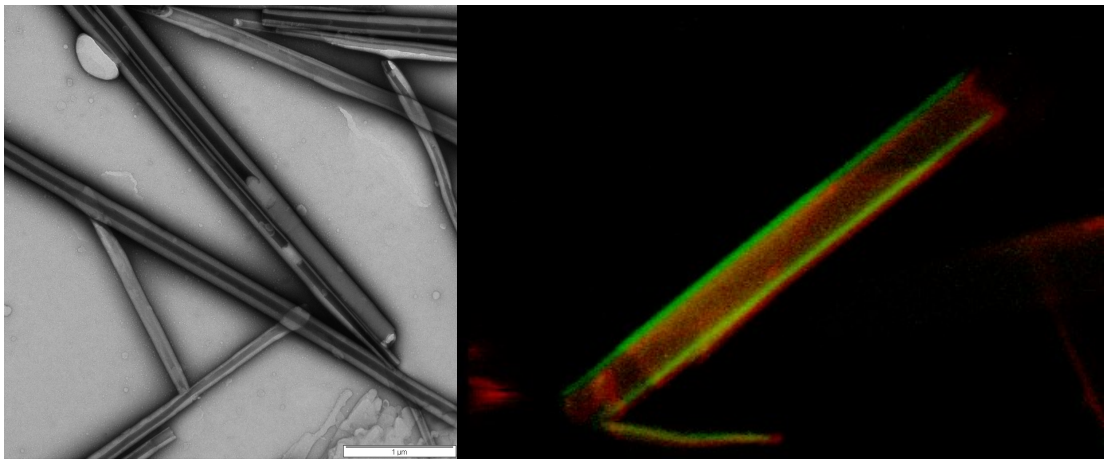


Figure S15. Left, TEM image of CholAU/DOPC nano-tubules loaded with contrast agent. Scale bar corresponds to 1 μm. Right, fluorescence confocal image of a CholAU/DOPC/NBD-DPPE micro-tubule loaded with rhodamine-dextran. Green and red correspond to NBD-DPPE and rodamine-dextran fluorescence, respectively. Scale bar corresponds to 5 μm.